DAIDS

VIROLOGY MANUAL

FOR HIV LABORATORIES

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Compiled by

THE DIVISION OF AIDS

NATIONAL INSTITUTE OF ALLERGY & INFECTIOUS DISEASES

NATIONAL INSTITUTES OF HEALTH

and

COLLABORATING INVESTIGATORS

CRYOPRESERVATION AND THAWING OF PBMC

I. REAGENTS

Ice

Cryoprotective Medium: RPMI 1640 containing glutamine, 10% sterile DMSO and 50% heat-inactivated fetal bovine serum (FBS). This medium should be prepared fresh for each freezing procedure and cooled to 2 to 8^0C prior to use.

Thawing Medium: RPMI 1640 containing 10% heat-inactivated FBS warmed to 20 to 24^0C.

Viability stain: 0.4% trypan blue solution, e.g.,, Sigma T8154.

Cryovials placed in ice.

II. FREEZING PROCEDURE

1. PBMC at a known concentration are centrifuged at 400 x g for 10 minutes at 20 to 24^0C and the supernatant is removed.

2. The PBMC are resuspended to a concentration of 2.5×10^6 to 1×10^7 PBMC/mL (keep on ice) with cold Cryoprotective Medium. The Cryoprotective Medium is added dropwise, with constant mixing, over 1 to 2 minutes.

3. Dispense 1 mL aliquots of the cell suspension into cryovials. Place the cryovials in a small, insulated (styrofoam) container in the bottom of a -70^0C freezer for 2 to 24 hours, then transfer to vapor-phase liquid nitrogen for storage.

III. THAWING PROCEDURE AND DETERMINATION OF VIABILITY

1. The frozen cells should be thawed rapidly in a 37^0C water bath until only a small crystal of ice remains. Cells must be handled gently to avoid mechanical injury.

2. The cells are transferred to a sterile 15-mL conical centrifuge tube following which Thawing Medium is added dropwise down the side of the tube, gently mixing with the cell suspension. Continue adding medium and mixing until the tube is filled.

3. The tube is centrifuged at 400 x g for 10 minutes at 20 to 24^0C and the supernatant is discarded. The cells are resuspended in coculture medium. An aliquot is removed and placed in PBS or Hanks balanced salt solution to yield

about 2 to 5 x 10^5 PBMC/mL. The viability is determined using 0.4% trypan blue solution. The viability should be greater than 80%.

IV. REFERENCES

Gjerset G, Nelson KA and Strong DM. Methods for cryopreserving cells, pp 61-67. *In* Manual of Clinical Laboratory Immunology, NR Rose, EC de Macario, JL Fahey, H Friedman and GM Penn (eds.), 4th ed., American Society for Microbiology, Washington D.C., 1992.